
 ABSTRACTS (MASTER THESIS)

Establishment of virus-induced gene silencing (VIGS) system by use of domestic plant viruses in *Lithospermum erythrorhizon*

**(Graduate School of Agriculture,
Laboratory of Plant Gene Expression, RISH, Kyoto University)**

Yuki Izuishi

Lithospermum erythrorhizon is a boraginaceae medicinal plant that produces a unique red naphthoquinone called shikonin as its ester derivatives. These pigments are accumulated in the roots of this herbal plant. The dried roots have been used as a crude drug in Asian countries, and shikonin derivatives are major active pharmaceutical components of this herbal medicine. Many divergent biological activities have been reported in shikonin and its stereo-isomer alkannin; i.e., conventionally, antibacterial, anti-inflammatory, anti-oxidant, antitumor, granulation enhancer, and more recently anti-angiogenic [1], anti-topoisomerase [2], increment of glucose uptake [3], and decrease in adiposity [4].

Biosynthetic pathway of shikonin were actively studied by biochemical methods using enzymatic analysis, and also by feeding experiments with radio-labeled compounds from 1970s, as summarized in a review [1]. Currently, synthetic biology approaches are actively studied to synthesize high value plant products in unicellular organisms, while the shikonin biosynthetic pathway is not completely clarified. There are many candidate genes paving the gap in the latter shikonin biosynthetic pathway, while an effective evaluation system for the gene functions is missing. RNAi-mediated suppression is only one feasible method for this, but it needs long period to evaluate the gene function after generating hairy roots, while virus-induced gene silencing (VIGS) system provides more rapid and efficient evaluation system as it provides an easy knockdown system by simple infection.

In this study, we tried several plant viruses as vectors to induce gene fragments, by which the functions of candidate genes are knocked down. As a model gene, we selected phytoene desaturase (*PDS*) gene to knock down in this plant, because the phenotype of VIGS is easily recognized by visual evaluations.

References

- [1] Hisa, T., Kimura, Y., Takada, K., Suzuki, F. & Takigawa, M. Shikonin, an ingredient of *Lithospermum erythrorhizon*, inhibits angiogenesis in vivo and in vitro. *Anticancer Res.* 18, 783-288 (1998).
- [2] Ahn, B., Baik, K., Kweon, G., Lim, K. & Hwang, B. Acylshikonin analogues: synthesis and inhibition of DNA topoisomerase-I. *J Med Chem.* 38, 1044-1047 (1995).
- [3] Oberg, A. *et al.* Shikonin increases glucose uptake in skeletal muscle cells and improves plasma glucose levels in diabetic Goto-Kakizaki rats. *PLoS One*, 6; 10.1371/journal.pone.0022510 (2011).
- [4] Bettaieb, A. *et al.* Decreased adiposity and enhanced glucose tolerance in shikonin treated mice. *Obesity (Silver Spring)*. 23, 2269-2277 (2015).